(ii) transferring said transfected PGCs into a recipient avian embryo of the same species as the avian used to obtain said isolated, purified PGCs; and

(iii) allowing said recipient avian to develop into a chimeric bird.

26. (New) An improved method of producing chimeric avians which comprises:

(i) isolating a population of transfected avian primordial germ cells according to the method of Claim 24;

(ii) transferring said transfected PGCs into a recipient avian embryo of the same species as the avian used to obtain said isolated, purified PGCs; and

(iii) allowing said recipient avian to develop into a chimeric bird.

27. (New) A transgenic chimeric avian isolated according to the method of Claim 25.

28. (New) A transgenic chimeric avian isolated according to the method of Claim 26.

REMARKS

This amendment is responsive to the Office Action dated October 1, 1999. Entry of the foregoing and favorable reconsideration of the subject application in light

of the following remarks, pursuant to and consistent with 37 CFR §1.112, are respectfully requested.

At the outset, Applicants acknowledge with appreciation the Examiner's noticing and renumbering the misnumbered claims. Because newly numbered claim 17 is now a duplicate of claim 16, claim 17 has been canceled. Applicants also note the amendments to the claims presented above. In particular, claim 1 has been amended to conform with the language used in the allowed claims of the parent application, and to incorporate the limitations of original claims 6 and 9. Accordingly, claims 6 and 9 have been canceled and the dependencies of claims 7 and 10 have been corrected such that claims 7 and 10 now depend on claim 1 rather the canceled claims. Claim 2 has been amended to recite the preferred minimum amounts of cofactors and finds support in the specification at page 20, lines 12-14. It is important to note, however, that lower amounts may be used depending on the cells and the other ingredients, and claim 2 as amended should not be construed to limit the quantities of cofactors employed. In addition, claim 11 has been amended to conform with the language used in the allowed claims of the parent application, and to incorporate the limitations of original claim 14. Accordingly, claim 14 has been canceled and the dependency of claim 15 has been corrected such that claim 15 depends on claim 11. Finally, claims 19 and 20 have also been amended to conform to the language employed in the claims previously allowed in the parent application.

Also, claim 21 has been canceled because it no longer further limits claim 19 in view of the amendment to claim 1. No new matter has been added by any of these amendments.

Also, new claims 22-28 have been added in order to emphasize the inventive contribution of the claimed invention. For instance, new claim 22 is directed to an improved method for transfecting avian primordial germ cells wherein said avian primordial germ cells are capable of being maintained for periods of at least fourteen days in tissue culture. This new claim is presented to emphasize the fact that, although transfection of avian PGCs was known in the art before the present invention, the present invention provides an advantage over the prior art in that it permits PGCs to be cultured for prolonged periods, which in turn allows for transfected cells to be more readily identified or selected. The goal of the present invention is discussed on page 7, lines 5-7, wherein it is stated, "to date, the growth of PGCs in culture for prolonged periods to facilitate selection of transfected PGCs has not been achieved."

In particular, new claim 22 is directed to an improved method comprising:

(i) isolating a pure population of primordial germ cells (PGCs) from an avian;

(ii) transfecting said PGCs with a desired nucleic acid; and

(iii) maintaining said PGCs in a tissue culture medium comprising at least the following growth factors:

(1) leukemia inhibitory factor (LIF),

(2) basic fibroblast growth factor (bFGF),

(3) stem cell factor (SCF) and

(4) insulin-like growth factor (IGF);

until transfected PGCs containing the inserted nucleic acid are identified. Claim 23 further specifies that the transfected nucleic acid encodes a therapeutic protein. Support for these new claims may be found in the claims as originally filed, and on page 16, lines 7-19. New claim 24 specifies that the transfected nucleic acid according to the improved method comprises a marker DNA, and that said transfected PGCs are selected by virtue of the expression of said marker DNA. Support for this claim may be found on page 17, lines 5-8. New claims 25 and 26 are directed to improved methods of producing chimeric avians using the transfected avian PGCs according to claims 22 and 24, respectively, and are improved over previous known methods of producing chimeric birds in that PGCs which contain the transfected gene may be identified or selected by virtue of expression of the therapeutic protein or expression of the marker gene before being transferred into a recipient avian embryo, thereby foregoing or decreasing the need to screen resulting birds for presence of the transgene, and increasing the probability that the resulting chimeric birds will express

the desired gene. Support for such claims may be found on page 17, lines 4-7 of the specification. Finally, claims 27 and 28 are directed to transgenic chimeric avians produced by the improved methods of claims 25 and 26, and find support on page 17, lines 9-13. No new matter has been added.

Turning now to the Office Action, claims 14-17 and 19-21 were rejected under 35 U.S.C. §112, first paragraph, because the specification allegedly fails to enable a method of making a chimeric avian which requires the use of a transfected PGC. In particular, the Examiner notes that the state of the art is not well established by Applicants' own admission on pages 3-5 of the specification, and that Applicants fail to show any working example of a chimeric bird made from a transfected PGC in the specification. Applicants respectfully traverse the rejection.

Firstly, Applicants note that claim 14 has been canceled because the limitations recited therein have been incorporated into claim 1. However, it is Applicants position that claim 1 as amended is enabled, and that the rejection is not appropriately applicable to amended claim 1 for the same reasons it is not applicable to original claim 14.

Applicants respectfully note that the discussion on pages 3-5 of the specification to which the Examiner refers concerned the deficiencies of the prior art methods, which, as Applicants specifically point out on page 5, lines 3-7, suffered in particular from the lack of long term culture methods for chicken ES and PGC cells.

The prior art had not failed in producing chimeric or transgenic avians (see specification, page 5, lines 12-19); rather, such avians were previously *difficult* to produce due to the lack of PGCs that could be cultured for prolonged periods. Thus, the goal of the present invention was not to produce a transgenic or chimeric avian per se as a novel invention. Rather, Applicants' goal was to develop a long term culture system for avian PGCs that would *facilitate* the production of transgenic and chimeric avians (see, for instance, the discussion in the specification at page 5, lines 9-11).

The fact that it was known in the art that transgenic chimeric birds could be made using transformed PGCs is evidenced by the attached article by Vick and Simkiss. According to the abstract, these authors isolated PGCs from avian embryos and transfected the PGCs using defective retroviruses. These transfected PGCs were then injected into recipient embryos to form chimeras, some of which were able to pass the foreign DNA on the their offspring. What this group did not show is the ability to culture the PGCs long term in culture prior to transfection, a technique that would *facilitate* transfection with a wide variety of vectors other than retroviruses, that would have allowed for selection of cells receiving the transfected DNA prior to chimera formation, which in turn would have increased the number and type of chimeric birds that could have been isolated using this technique.

Thus, the Examiner has rejected claims directed to methods of making transgenic chimeric avians because of deficiencies in the prior art which Applicants

have in fact overcome. Moreover, the Examiner has rejected claims directed to such methods on the basis that no transgenic chimeric avians were demonstrated in a working example, when in fact such animals had been made before, just with much more difficulty in the absence of the present invention, which provides for long term culture of avian PGCs.

In this regard, the Federal Circuit has long held that 35 USC §112 does not require a specific teaching of that which is already known to one of ordinary skill in the art. Case v. CPC International, Inc., 221 USPQ 196, 201 (Fed. Cir. 1984). Moreover, it is well-established that the "absence of working examples in specification is without significance, since examples are not necessary and even though [they] may provide added useful information, the test is whether individual possessed of knowledge of one skilled in the art could practice invention without exercise of undue amount of experimentation." Ex Parte Nardi & Simier, 229 USPQ 79, 80 (Bd. App. 1986).

The Examiner makes the argument at the bottom of page 3 of the Office Action that it would require a "large amount of experimentation" for successful transfection in view of the fact that Applicants reported that only 1 in 50 PGCs was transfected. But this is true for any transfection employing a new cell line or a new vector construct, and in fact was true of the prior art techniques. In this regard, even the Examiner notes on page 9 of the Office Action, paragraph 12, that "one of skill in the

art would readily know how to transfect PGCs." The fact that a "large amount of experimentation" may be necessary is not fatal because the standard for enablement is whether the experimentation required is *undue*.

As discussed above, prior persons of ordinary skill were not deterred from making transgenic chimeric avians even though they could not culture PGCs more than a few days, even though they could not confirm or select transfected PGCs before they proceeded with injection of the cells, and even though they had to go through the entire process of hatching the chimeric birds and mating them to see if the transfection enabled germ line transmission. The fact that persons using prior art techniques still sought to make transgenic chimeric birds despite the fact that they did not have the benefit of Applicants' invention suggests that, even in this uncertain environment, the level of experimentation was not undue. Applicants fail to comprehend, then, how the presently claimed methods of making transgenic chimeric avians would require "undue" experimentation when the claimed methods only make the prior art methods easier, and the level of experimentation was not so inconceivably large to deter prior researchers from making transgenic chimeric birds in the absence of the benefits to be gained by Applicants' invention.

Applicants respectfully request reconsideration and withdrawal of the rejection of claims 14-17 under §112, first paragraph in view of the above comments, and further request that the rejection not be applied to the amended claims.

Before moving on in the Office Action, Applicants note that claims 19-21 directed to a PGC cell line were also included in the rejection under § 112, first paragraph, and on page 4 of the Office Action it is alleged that the instant specification does not enable one of skill in the art to make or use a PGC cell line. Without necessarily agreeing with this rejection (because indeed, as discussed at the bottom of page 14 of the specification, cells have been cultured in the disclosed media of the invention for up to four months), claims 19-21 have been amended to conform with the language employed in the allowed claims of the parent application in order to expedite prosecution and are now directed to a culture of isolated PGCs rather than a PGC cell line per se. Withdrawal of the rejection of claims 19-21 under §112, first paragraph, is therefore respectfully requested.

Next, claims 1-21 were rejected under 35 U.S.C. §112, second paragraph for alleged indefiniteness. The rejections are addressed in the order that they were presented in the Office Action for the convenience of the Examiner.

First, claim 1 was rejected because of the terminology "prolonged periods." Because this phrase was deleted by amendment above, the rejection appears to be moot and withdrawal is respectfully requested.

Next claims 10, 15, 16 and 17 were rejected for the phrase "therapeutic polypeptide" because it is allegedly unclear whether the polypeptide is therapeutic to the resultant chicken, or someone ingesting an egg, or is supposed to be produced by

the chicken or egg for use in a polypeptide composition. Applicants respectfully traverse the rejection.

Applicants first note that the specification indeed discusses the utility of the transgenic and chimeric eggs and chickens of the invention at page 17, lines 10-13, where it is stated: "Preferably, the desired protein will then be recovered from the eggs of these transgenic avians, thereby providing a continual supply of the protein. Alternatively, the protein may be recovered from chimeric birds directly, e.g., isolated from the circulatory system." Thus, when the claims are read in light of the disclosure, it is clear that one use of the transgenic eggs and chickens of the present invention is to provide an expression system for useful proteins. In this regard, it has long been understood by those having any skill in the art that the recombinant production of proteins enables an alternative environment from which to isolate a protein of interest, and often provides much more protein, or a more ready source of protein, than if the protein were to be isolated from its natural environment.

Thus, the specification discusses the utility of the present methods for providing a continuous supply of a protein of interest. Considering that the common definition of "therapeutic" is "having or exhibiting healing powers," [1] it would be quite clear to one having ordinary skill in the art upon reading the claims in light of the disclosure that the invention would encompass the production of "therapeutic"

[1] See The American Heritage College Dictionary, 3rd ed. (1997), page 1406.

proteins, or those having medicinal value, using the avian expression system described. However, it should be noted that the uses of the eggs and avians produced by the present invention are limited only by one's imagination. Indeed, the Examiner has noticed that the eggs could also be eaten directly by one in need of exposure to a therapeutic protein, and that the invention could also be employed to express proteins that are beneficial to the avian in which they are produced. The present methods, however, are not directed to methods of using the eggs and avians of the invention, but rather to making them. As such, a single disclosed use is sufficient (although many more are indeed possible).

In view of these comments, reconsideration and withdrawal of the rejection of claims 10, 15, 16 and 17 is respectfully requested.

Claim 11 was rejected for the phrase "desired PGC phenotype" and the lack of antecedent basis therefor. Claim 11 was amended above to clarify that the desired phenotype was that conferred by the nucleic acid transfected into the PGC, and to resolve the issue of antecedent basis. Accordingly, this rejection is now moot and should be withdrawn.

On page 6, of the Office Action, the Examiner indicated that claim 17 would be rejected for double patenting if claim 16 was allowed, in view of the fact that claim 17 is a duplicate of claim 16. Claim 17 has been deleted by way of amendment above, therefore, this double patenting is no longer a potential issue.

Claims 1-6, 11-13 and 18 were provisionally rejected for obvious-type double patenting as being allegedly unpatentable over claims in parent application 08/905,773. Because the substance of the claims could change through the course of prosecution, Applicants respectfully request that this rejection be held in abeyance until the claims are otherwise in condition for allowance. If the obvious-type double patenting rejection remains at that time, Applicants will gladly submit a terminal disclaimer.

Finally, claims 1-13 and 18 were rejected under 35 U.S.C. §103(a) as being unpatentable over Pain et al. While certainly not agreeing with the rejection (in fact, this reference as applied to claims without the transfection step was overcome in the parent application), Applicants respectfully note that claims 1 and 11 have been amended above to incorporate the limitation of claim 14, which the Examiner states on page 9 of the Office Action, paragraph 12, is free of the prior art of record. Therefore, amended claims 1 and 11 must be free of the prior art of record. Moreover, because claims 2-13 and 18 are each directly or indirectly dependent on either claim 1 or claim 11, all these claims must also be free of the prior art of record. Accordingly, the rejection has been rendered moot, and should now be withdrawn.

In view of the above amendments and remarks, this application is believed to be in allowable condition. If there are any questions regarding this amendment and response, or with the application in general, the Examiner is respectfully requested to telephone the undersigned so that prosecution of the present application may be expedited.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By: Bonnie D Weiss

Bonnie D. Weiss
Reg. No. 43,255

by Reg # 35,030

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